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(54) Title: NON-ISOTOPIC DETECTION OF NUCLEIC ACID SEQUENCES USING AN RecA LABEL

(57) Abstract

Method to detect single-stranded nucleic acid by contacting a solid phase bonded with the nucleic acid with a single-stranded DNA probe including RecA protein, and detecting the presence of RecA protein bound with the probe-single-stranded nucleic acid hybrid complex.

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DESCRIPTION

Non-Isotopic Detection of Nucleic Acid Sequences Using a RecA Label

Background of the Invention

Weinstock et al., 76 Proc. Natl. Acad. Sci. USA 126, 1979, McEntee, 24 Biochemistry 4345, 1985, and Bryant and Lehman, 82 Proc. Natl. Acad. Sci. USA 297, 1985, describe the mechanism of renaturation of complimentary DNA strands by the RecA protein of *Escherichia coli*.

Honigberg et al., 83 Proc. Natl. Acad. Sci. USA 9586, 1986, describe the ability of RecA protein to promote formation of base pairing between single-stranded DNA and duplex DNA. Radding et al., U.S. Patent No. 4,888,274 and PCT Application WO 87/01730 described methods for use of this phenomenon for isolating specific duplex nucleic acids.

Summary of the Invention

This invention features use of RecA-coated single-stranded DNA probes for non-isotopic detection of target sequences of single-stranded DNA or RNA immobilized on a solid support. Applicant describes the use of RecA coated single-stranded DNA which can specifically hybridize to single-stranded target sequences, and the use of the RecA as a label to allow specific detection of hybrids formed between the single-stranded DNA probe and the target nucleic acid.

Thus, in a first aspect the invention features a non-isotopic method for detection of a single-stranded nucleic acid by contacting a solid phase bonded with the nucleic acid with a single-stranded DNA probe bonded with a RecA protein, and detecting the presence of RecA protein bound with the probe-single-stranded nucleic acid hybrid complex.

In preferred embodiments, the RecA protein is bonded to the single-stranded DNA probe in the presence of magnesium ions and ATPγs, and the RecA protein is covalently bonded with an enzyme label, or some other readily detectable label which can be bonded with the RecA protein.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

The drawing will first briefly be described.

Drawing

The figure is a diagrammatic representation of a method of the invention.

Specifically, the present invention relates to the use of RecA-coated single-stranded DNA probes for the non-isotopic detection of target sequences of DNA or RNA immobilized on a solid support. To practice the invention, one first obtains a probe DNA molecule for the target sequence of interest using standard methods. This probe is then reacted with either RecA protein in the presence of 1-2 mM ATPγs or with an enzyme conjugate of RecA protein, such enzymes preferably being alkaline phosphatase or horseradish peroxidase. These conjugates may be prepared using standard methods in which both proteins are activated with appropriate reagents and coupled under controlled conditions such that the activities of both are retained. Alternatively, one can isolate a fusion protein of RecA with an enzyme, e.g., alkaline phosphatase, or its equivalent by appropriate manipulation of coding sequences for the genes of the two proteins. This protein could then be reacted with the single-stranded DNA in the presence of ATPγs to generate the coated probe.

The probe coated with RecA protein or the RecA-conjugate is allowed to react, in the presence of an appropriate buffer containing Mg++ and ATPγs, with denatured DNA or RNA immobilized on a solid support, preferably a nylon or nitrocellulose membrane. The DNA or RNA contains the target sequence of interest along with other unrelated sequences.

The probe will bind to the target sequence by complementary base pairing, and excess probe is washed off the membrane. The presence of the bound probe-RecA-target complex may be detected by reacting it with a labeled or unlabeled antibody against RecA, e.g., an antibody conjugated to alkaline phosphatase or horseradish peroxidase, and then adding a substrate for alkaline phosphatase or horseradish peroxidase to generate either a color signal, or a chemiluminescent signal detectable with standard X-ray film as a dark band. If a conjugate of RecA protein or a RecA-enzyme fusion protein is used, one need only add a substrate for the enzyme and detect the color or chemiluminescent signal by standard methods, thereby avoiding the antibody step.

Other embodiments are within the following claims.

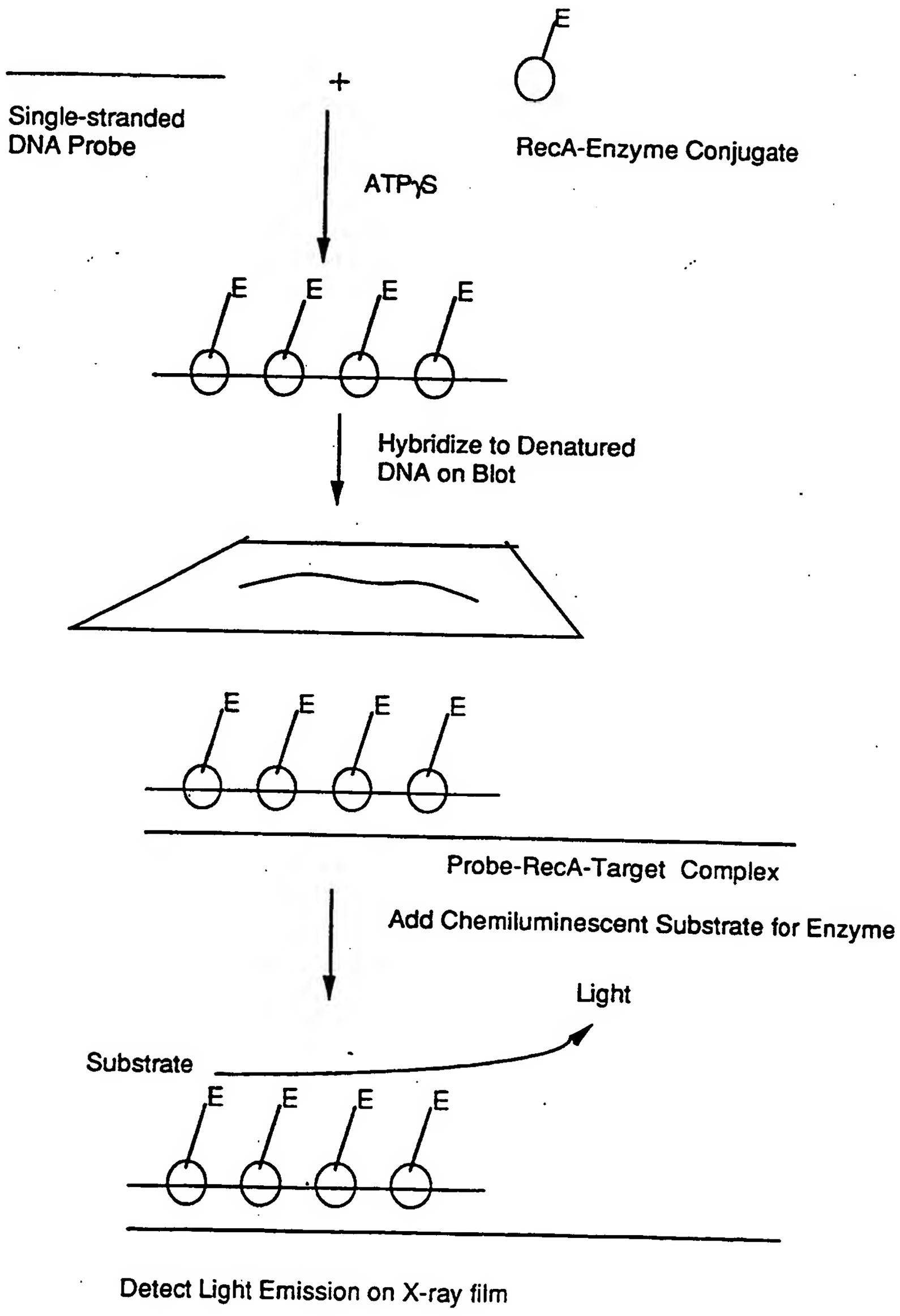
Claims

1. A method for non-isotopic detection of a single-stranded nucleic acid, comprising contacting a solid phase bonded with said nucleic acid with a single-stranded DNA probe comprising RecA protein, and

detecting the presence of RecA protein bound to said single-stranded nucleic acid.

2. The method of claim 1, wherein said contacting is in the presence of magnesium ions and ATPγs.

3. The method of claim 1, wherein said RecA protein is covalently bound to an enzyme.



Detect Light Emission on X-ray film

INTERNATIONAL SEARCH REPORT

Int[illegible]ional application No.
PCT/US93/07150

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12Q 1/68
US CL :435/6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 935/77, 78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, CHEM ABSTRACTS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,011,770 (KUNG ET AL.) 30 APRIL 1991, SEE ENTIRE DOCUMENT.	1-3
Y	US, A, 4,888,274 (RADDING ET AL.) 19 DECEMBER 1989, SEE SUMMARY OF THE INVENTION, COLUMNS 2-4.	1-3
Y	WO, A, 85/05685 (ZAPOLSKI ET AL.) 19 DECEMBER 1985, SEE PAGES 1-5.	1-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer MARGARET PARR Telephone No. (703) 308-0196

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